CHIMERIC INFLUENZA VIRUS-LIKE PARTICLES COMPRISING HEMAGGLUTININ

This application claims priority from U.S. Provisional Application No. 61/220,161 filed Jun. 24, 2009.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The content of the electronically submitted substitute sequence listing, file name 3043_0020001_SequenceListing.ascii, size 256,991 bytes; and date of creation Aug. 20, 2013, filed herewith, is incorporated herein by reference in its entirety.

FIELD OF INVENTION

The present invention relates to virus-like particles. More specifically, the present invention is directed to virus-like particles comprising chimeric influenza hemagglutinin, and methods of producing chimeric influenza virus-like particles.

BACKGROUND OF THE INVENTION

Influenza is the leading cause of death in humans due to a respiratory virus, and during "flu season", it is estimated that 10-20% of the population worldwide may be infected, leading to 250-500,000 deaths annually.

The current method of combating influenza in humans is by annual vaccination. The vaccine is usually a combination of several strains that are predicted to be the dominant strains for the coming flu-season, however the number of vaccine doses produced annually is not sufficient to vaccinate the world's population. For example, Canada and the United-States obtain enough vaccine doses to immunize about one third of their population, and in Europe, only about 17% can be vaccinated given current production—in the face of a worldwide flu pandemic, this production would be insufficient. Even if the necessary annual production could somehow be met in a given year, the dominant strains change from year to year, thus stockpiling at low-need times in the year is not practical. Economical, large scale production of an effective influenza vaccine is of significant interest to government and private industry alike.

Influenza haemagglutinin (HA) surface glycoprotein is both a receptor-binding and membrane fusion protein. It is a trimer of identical subunits, each containing two disulphide-linked polypeptides, HA1 and HA2, that are derived by proteolytic cleavage of a precursor, HA0, that has a signal peptide sequence at its N-terminus and a membrane anchor sequence at its C-terminus. Cleavage to form HA1 and HA2 generates the N-terminus of the smaller polypeptide, HA2, which has the membrane anchor sequence at its C-terminus. Cleavage is required for membrane fusion activity but not for immunogenicity. The HA2 N-terminal sequence is called the 'fusion peptide' because cleavage at similar hydrophobic sequences is also required for the activity of other virus fusion proteins, and because 20-residue synthetic peptide analogues of the sequence fuse membranes in vitro.

Generally, the surface of the globular 'head' comprises several flexible loops with well-characterized and variable antigenic regions designated as sites A, B, C, D and E (reviewed in Wiley et al., 1987. Annu. Rev Biochem 56:365-394). Insertion or replacement of short peptide sequences at some sites (e.g. B and E) for immunity studies have been described (Garcia-Sastré et al. 1995. Biologicals 23:171-178). Epidermal growth factor (EGF), single chain antibody (scFV) and the Fc domain of an IgG, ranging in size from 53 to 246 amino acids, have been inserted at the N-terminal end of a H7 and chimeras has been successfully expressed (Hatziioannou et al., 1999. Human Gene Therapy 10:1533-1544). More recently, 90 and 140 amino acid domains of *Bacillus anthracis* protective antigen have been fused to the amino terminus of a H3 (Li et al., 2005. J. Virol 79:10003-1002). Copeland (Copeland et al., 2005. J. Virol 79:6459-6471) describes the expression of the gp120 Env HIV surface glycoprotein on a H3 stalk, where the gp120 domain replaced the whole globular head of HA.

Several recombinant products have been developed as recombinant influenza vaccine candidates. These approaches have focused on the expression, production, and purification of influenza type A HA and NA proteins, including expression of these proteins using baculovirus infected insect cells (Crawford et al, 1999 Vaccine 17:2265-74; Johansson, 1999 Vaccine 17:2073-80), viral vectors, and DNA vaccine constructs (Olsen et al., 1997 Vaccine 15:1149-56).

Production of a non-infectious influenza virus strain for vaccine purposes is one way to avoid inadvertent infection. Alternatively, virus-like particles (VLPs) as substitutes for the cultured virus have been investigated. VLPs mimic the structure of the viral capsid, but lack a genome, and thus cannot replicate or provide a means for a secondary infection. Current influenza VLP production technologies rely on the co-expression of multiple viral proteins, and this dependence represents a drawback of these technologies since in case of a pandemic and of yearly epidemics, response time is crucial for vaccination. A simpler VLP production system, for example, one that relies on the expression of only one or a few viral proteins without requiring expression of non-structural viral proteins is desirable to accelerate the development of vaccines.

Enveloped viruses may obtain their lipid envelope when 'budding' out of the infected cell and obtain the membrane from the plasma membrane, or from that of an internal organelle. In mammalian or baculovirus cell systems, for example, influenza buds from the plasma membrane (Quan et al 2007 J. Virol 81:3514-3524). Only a few enveloped viruses are known to infect plants (for example, members of the Tospoviruses and Rhabdoviruses). Of the known plant enveloped viruses, they are characterized by budding from internal membranes of the host cell, and not from the plasma membrane. Although a small number of recombinant VLPs have been produced in plant hosts, none were derived from the plasma membrane, raising the question whether plasma membrane-derived VLPs, including influenza VLPs can be produced in plants.

Formation of VLPs, in any system, places considerable demands on the structure of the proteins—altering short stretches of sequence that correspond to selected surface loops of a globular structure may not have much of an effect on expression of the polypeptide itself, however structural studies are lacking to demonstrate the effect of such alterations on the formation of VLPs. The cooperation of the various regions and structures of HA (e.g. the membrane anchor sequences, the stalk or stem regions of the trimer that separate the globular head from the membranes) has evolved with the virus and may not be amendable to similar alterations without loss of HA trimer integrity and VLP formation.